Long Range RNA-RNA interactions with the genome of classical swine fever virus

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Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

LONG RANGE
RNA-RNA INTERACTIONS

within the genome of classical swine fever virus

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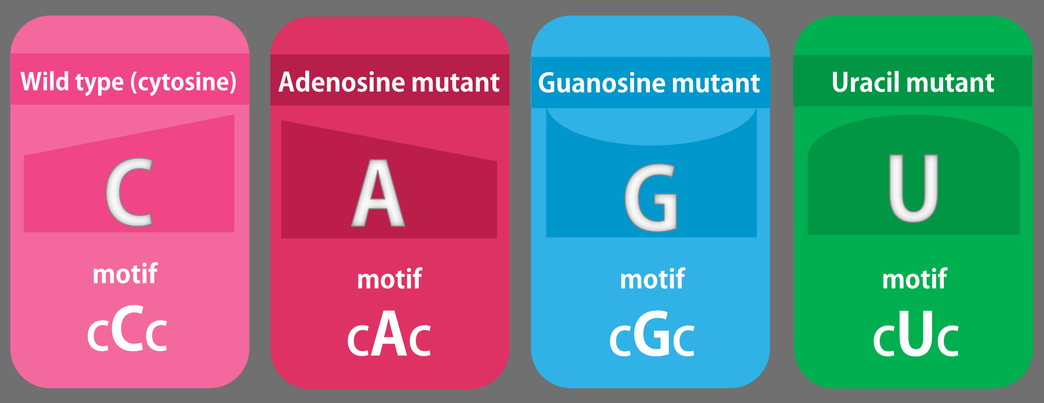
INTRODUCTION

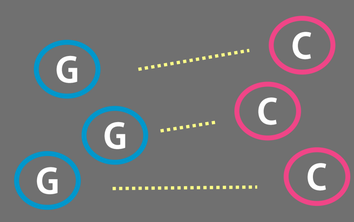
Classical swine fever (CSF) is a highly contagious porcine disease caused by classical swine fever virus (CSFV). The 5'-untranslated region (5'UTR) of the CSFV RNA genome contains an internal ribosomal entry site (IRES) directing the cap-independent initiation of protein synthesis. Besides IRES function, the 5'UTR may also contain *cis*-acting RNA elements that are important not only for viral translation and replication but also important in regulating the switch between these processes.

A motif in the coding region for NS5B (the RNA polymerase) has now been identified in the CSFV RNA which might interact with the IRES subdomain $IIId_1$.



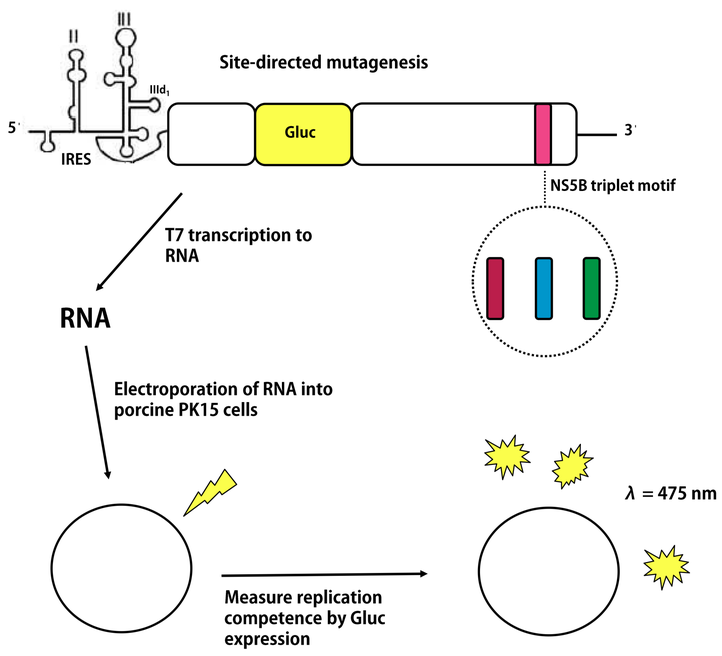
NS5B mutants with modifications in a triplet CCC motif

To investigate whether this putative interaction in the CSFV RNA is important in viral replication, a triplet motif of three cytosines (CCC) spanning two codons (xCC | Cxx) within the NS5B coding region was modified to affect the possible interaction with the IRES domain $IIId_1$ that contains a GGG motif.



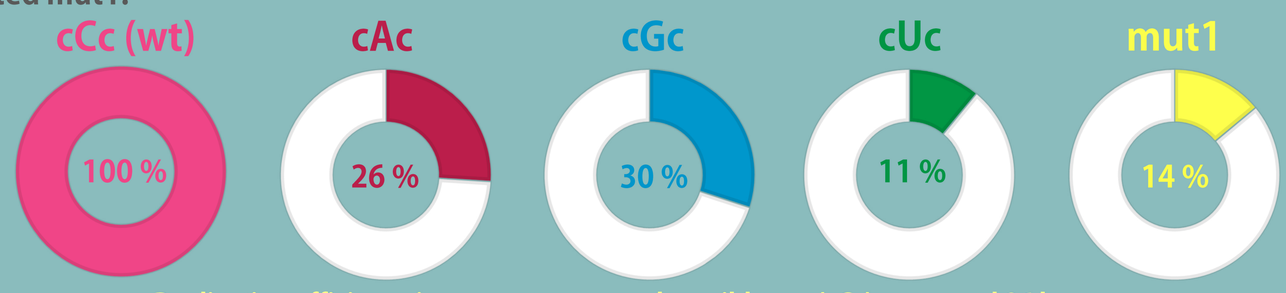
METHODS

By site-directed mutagenesis, specific silent mutations have been introduced into the triplet motif within the NS5B sequence and the effect on replication has been analysed using a luciferase reporter system.

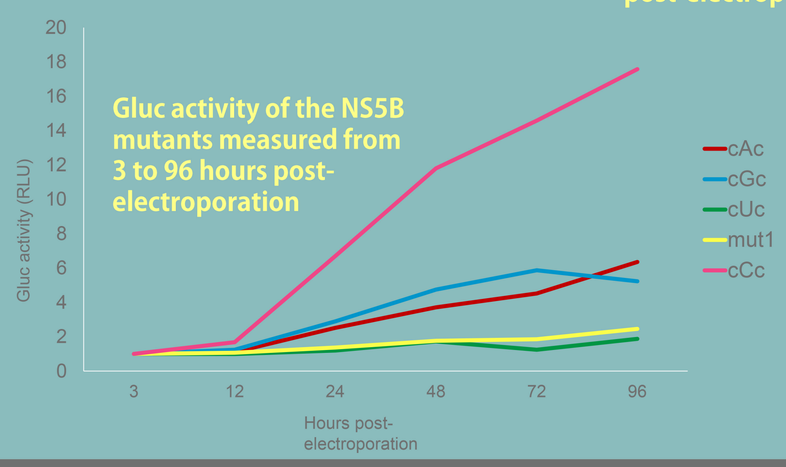


RESULTS

All three NS5B mutants have reduced viral replication efficiency. In particular, the mutant with a cytosine to uracil change (resulting in no amino acid change within the protein), exhibited a substantial decrease in replication. This level is comparable to a replication defective mutant denoted mut1.



Replication efficiency in percent compared to wild type (cCc) measured 96 hours post-electroporation



The reduced replication capability indicates that the cCc motif is required for efficient viral replication. This effect is not associated with any change in the amino acid sequence of the RNA polymerase but likely due to interactions within the RNA genome.

Take home messages...

1 **A TRIPLET CCC MOTIF** in the NS5B coding region appears to be important for viral replication

2 **REDUCED REPLICATION EFFICIENCY** is observed after mutating the triplet motif (cAc, cGc, cUc) without changing the encoded protein sequence

3 **UNDERSTANDING PUTATIVE LONG RANGE INTERACTIONS** will provide valuable insight into the mechanisms underlying viral RNA replication

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